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DEMONSTRATE EXISTENCE OF ZnuABC GENES IN UROPATHOGENIC E.COLI CFT073 ISOLATED FROM INFECTED SEMEN

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ABSTRACT

Although the level of zinc in semen was exceedingly reduced during infection, E.coli remains survives actively via exploited even minute amount of zinc in medium for continuous their vitality by use ZnuABC system to uptake this depleted element from their environment. Thus, in order to verify and prove existence the gene that encoded this transporter, the PCR technique was applied in current study to achievement this mission accurately.

KEYWORDS: ZnuABC, zinc (Zn), E.coli, Harboring Bacterial Infections

INTRODUCTION

Most infectious diseases are accompanied by changed levels of several trace elements. However, sequential changes in trace elements in tissues harboring bacterial infections have not been studied. The host's normal response to infection (the acute phase response) includes elevated synthesis of metal-binding proteins and a concomitant flux of trace elements between blood and tissues (Beisel, 2004**)**. The most consistent responses involve a decrease in plasma levels of zinc (Zn) (Ilback *et al*., 2004). A decreased Zn can also be used to indicate infection before the development of clinical disease (Ilback *et al*., 2003).

Again, Several experimental studies, both in laboratory animals and adult human volunteers, have consistently found a systemic infections that introduced acute phase response cause dropped in the plasma zinc concentration, in other hand, a fall in plasma zinc concentration appeared shortly after the onset of a wide range of febrile infections (Falchuk, 1977). These changes are associated with simultaneous hypoferremia, hypercupremia, and elevations of selected plasma proteins, such as C-reactive protein (CRP) and α1-antitrypsin. Recently, reported that the acute phase response is mediated by cytokines such as interleukin 1 (IL-1) and tumor necrosis factor-α (TNF-α), which are secreted in response to infection (Cousins and Leinart, 1988; Van der Poll and Suaerwein, 1993). Thereafter, activate hepatic synthesis of metallothionein (MT), an intracellular metal-binding protein contributes to redistribution of zinc in the body (Schroeder and Cousins, 1990; Rofe *et al*., 1996). Relevant to, there are several proteins might involved in zinc homeostasis in Gram's negative bacteria have been identified in recent years. These protein was activated under conditions of zinc depletion, adequate zinc recruitment is ensured by the high affinity Zn- uptake system ZnuABC encoded by the *znuABC* genes, which were initially reported in *E. coli* (Ammendola *et al*., 2007). Genetic and biochemical studies have shown that ZnuABC expression was repressed in cells containing adequate amount of zinc. A few investigations carried out with different bacterial species have suggested that the integrity of the *ZnuABC* operon is essential to ensure the capability of various bacteria to grow in media devoid of zinc or within the infected host (Mourad *et al*., 2009).

MATERIALS AND METHODS

A total one hundred semen samples were collected from patients who attended to Fertility Center in AL Sadder teaching hospital in Al najaf city-Iraq at a period from June 2013 to Jan 2014, the ages of patients and control ranged from (18-55) years .The control samples were involved 20 healthy men.

Samples collection: the seminal fluid samples were collected from patients and control after informed them how to obtain the sample by sterile method into sterile container to avoid external contaminants. Then, the samples were cultured onto suitable media before preceding the next step to prevent contaminations, which may be occurring in next steps. After completion liquefactions period, the specimens were centrifuged at 3000 rpm for 15 minutes to obtain seminal plasma, then the concentration of zinc were estimated by using atomic absorption spectrometer technique. In order to verify and prove existence the gene that encoded this transporter, the PCR technique was applied in current study to achievement this mission accurately. The sequence and amplicon size of primer were applied in this study showed in table 1

Table 1: Primer Pairs Sequence and Amplicon Size

Gene	Primer Sequence (5′-3′)	Amplicon Size (bp)	Reference
Znu B	**Forward** **GTGCTGAACAACTGGGTATCT** **Reverse** **CTGTGCGCCATAATCCCTAATA**	Amplicon size (bp) 366	This Study

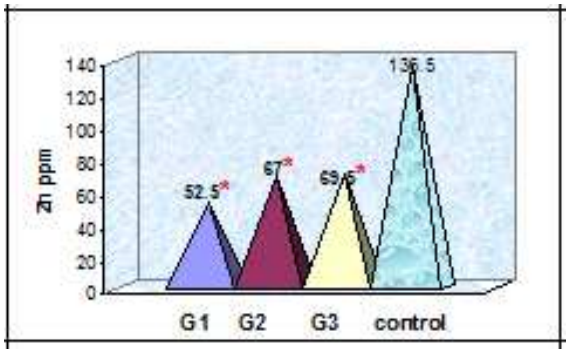
RESULTS AND DISCUSSIONS

In present study, the culturing of semen samples were yielded that the Gram's positive bacterial isolates were found in 41% of samples, while Gram's negative bacteria were constituted about 16%, the other 43% of samples have no growth, also our results indicate that the Escherichia coli is the most prevalent bacteria could be isolated from seminal fluid infection were constituted 22.8% out of all isolated bacteria. According to type of bacteria, the patients divided into three groups as shown in table 2

Table 2: Patients Groups of Study

Groups	Describe	Number
Group 1 (G1)	Gram negative infected patients	16
Group2 (G2)	Gram positive infected patients	41
Group3 (G3)	Uninfected subfertile patients	43
Group5 (G4)	Control (Healthy persons)	20

With regard to the concentration of Zinc, among overall groups, Mean ± SE G1 52.5 ±7.1, G2 67.7 ±6.5, and G3 69.57±5.3 have significant decreased ($P<0.05$) in contrast with control group 136.5 ±10.5 Figure 1.



***= P value <0.05**

Figure 1: Concentration of Zn ppm among Patients Groups Control

Therefore, this results are fully agreement with previous studies were reported that the infection considered as one of various factors (other than zinc intake) which influence concentration of zinc, thus, abroad range of febrile infections and/ or endotoxins can drive to reduction in this element (Baisel *et al*., 1973; Falchuk, 1977; Sugarma *et al*., 1982; Baisel, 1995).

These changes are associated with simultaneously hypoferremia, hypocupremia, and mounting of selective acute phase proteins as CRP and haptoglobin. In general, it was not clear to what extent of availability of zinc affects in vivo growth of infectious agents themselves. Notably, most microorganisms are required sufficient of zinc amount for essential cellular mechanisms (Bess *et al*., 1992; Sohnle *et al*., 1996). During infection, zinc element were redistributed mainly due to acute phase response, where transported from body fluid to liver and lymphocytes by means of a hepatocyte produced protein was called Metallothionein (MT), which exceed synthesis and secreted in response to IL-1 and TNF-α (Cousins and Leinari, 1988; Van der Poll and Suaerwein, 1993).

It has been believed that this state is an adaptive response intended to disturbed zinc of invading pathogens (Clohessy and Golden, 1996). However, reducing zinc concentrations are generally considered insufficient for bacterial vitality, hence, a highly localized suppression of extracellular zinc to microbiostatic concentrations may be created via acute phase reactants combining with calprotectin, which is a zinc binding protein introduced by PMN leukocytes (Clohessy and Golden, 1995).

Molecular Detection of ZnuB Gene in *E. coli- CFT073* by PCR

Beside the detection of *ZnuABC* genes of *E.coli* strain *CFT073* (*Uropathogenic E.coli*) in which considered the most predominant pathogenic bacteria were isolated in present study, thus, the existent of this gene have been explained how this bacterial strain can tolerates and survived in zinc deficient environment (e.g. semen after infection the zinc level were reduced) hence, possessing this gene enabled bacteria to survived in that niche.

Indeed, the high affinity ZnuABC system is considered as a crucial factor for pathogenesis of UPEC, and in spite of consists of three subunits ZnuA, ZnuB, and ZnuC, each of them encoded by separated gene (*ZnuA*, *ZnuB*, and *ZnuC* respectively), but the present study were dealt with only *ZnuB* gene for discrimination whole ZnuABC gene existence. Selection of *ZnuB* gene rather than *ZnuA* or *ZnuC*, may be due to the ZnuA has a pronounced effect on the equilibrium concentration of zinc in periplasmic space via pulling zinc from other metalloproteins, such as Cu/Zn superoxide dismutase, the alteration of this process enabling non specific divalent metals importers, this lead to misinterpretation of results, a part from, ZnuC probably encoded in other kinds of bacteria, thus choice of *ZnuB* gene is

more productive. Moreover, due to the ZnuB primer that designed and applied in present study is a highly specific for *E.coli CFT073* (Gunasekera etal., 2009) so, it's regarded as an excellent way to identifying this strain. In present study the primer was used in detection of *E.coli CFT073* were targeted the ZnuB subunit protein which existed in bacterial cytoplasmic membrane.

The results of this experiment were showed that a thirteen isolates of already identified *E.coli* were exhibited positive results for ZnuB gene Figure 2.

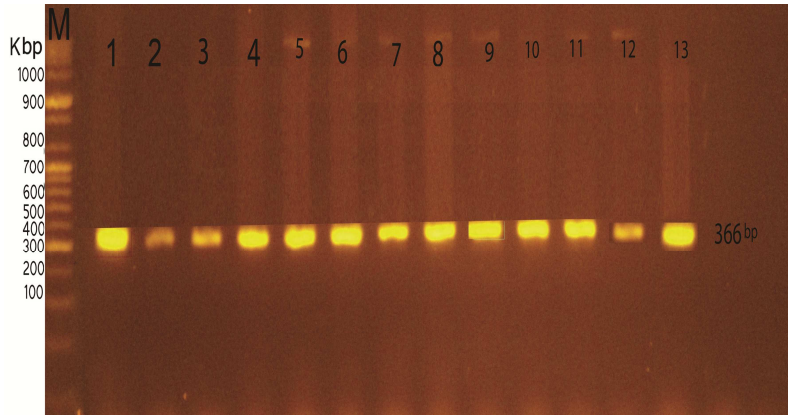


Figure 2: Gel Electrophoresis to Detect ZnuB Gene in Isolated *E.coli*

Ethidium bromide stained 1.5% agarose gel for I hour avd 100V showing PCR amplification product with 366bp primer for ZnuB gene in *E.coli*. Lane (M): DNA ladder (1000Kbp) standard size reference marker. Lane1, 2, 3, to Lane 13 showed *E.coli* CFT073 *ZnuB gene* had positive results.

Apart from, it is also notice that very high zinc levels could be microbicidal activity, for this reasons, the invaded microorganisms were evolved a certain auxiliary components could required for bacterial zinc homeostasis within intracellular enviroments, that facilitate metal recruitment throughout severe zinc depletion (Petrarca *et al*., 2011) and also probably contributed in the virulence of this bacteria (Campoy *et al*., 2002; Ammendola *et al*., 2007; Gabblanelli *et al*., 2011).

This outcomes agreement with (Patzer and Hantke, 1998; Gabbianelli *et al*., 2011), where report existence of high affinity ZnuABC system in *E.coli* will contribute to facilitate their adhesion to host epithelial cells, then establish successful infection, in addition to could be considered as a good marker for identification of UPEC.

CONCLUSIONS

From this study we can conclude, to enhance progressive survive and pathogenecity within zinc deficient medium like in genital infection, the invaded bacteria should possess certain mechanisms (e.g. ZnuABC system) enabling their growth in that environments

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